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(54) Title: IRAK3 POLYPEPTIDES, POLYNUCLEOTIDES AND METHODS

(57) Abstract

The invention provides methods and compositions relating to a novel kinase, IRAK3. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IRAK3 encoding nucleic acids or purified from human cells. The invention provides isolated IRAK3 hybridization probes and primers capable of specifically hybridizing with the disclosed IRAK3 genes, IRAK3–specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

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*IRAK3 Polypeptides, Polynucleotides and Methods*

## INTRODUCTION

Field of the Invention

The field of this invention is enzymes involved in signal transduction.

Background

Interleukin 1 (IL-1) receptor associated kinase (IRAK) functions as an intracellular signal transducer for the pro-inflammatory cytokine IL-1. IL-1 treatment of cells induces the complex formation of the two IL-1 receptor chains, IL-1R1 and IL-1RAcP, which recruits an adaptor molecule designated as MyD88 which binds to IRAK. IRAK is subsequently phosphorylated, released from the receptor complex to interact with TRAF6. TRAF6 triggers either the NIK/IKK kinase cascade to activate the transcription factor NF-κB or an undefined kinase cascade to activate the transcription factor AP-1. Both transcription factors regulate large numbers of genes that regulate immune and inflammatory responses.

The genome project has facilitated the identification of a large number of membrane bound receptor-like molecules that are related to IL-1RI and IL-1RAcP by sequence homology. One member of this family, IL-1RrP, has been recently shown to function as a receptor of an IL-1 related cytokine, IL-18, that regulates immune response by promoting the production of interferonγ. Like IL-1RI and IL-1RAcP, IL-1RrP signals NF-κB activation. Gene disruption experiments and biochemical analyzes indicate that IL-1RrP also utilizes MyD88 and IRAK and TRAF6 as intracellular signal transducers.

Although MyD88 deficient mice failed to respond to IL-1 and IL-18, IRAK deficient mice still have a residual response to IL-1. This observation indicates that other molecules in the cells can partially substitute for the function of IRAK in IL-1 signaling. Recently, an IRAK-related molecule designated IRAK2, was described. Although upon over expression, IRAK2 could interact with IL-1R and IRAK, it has not been shown to be recruited to the receptor complex after IL-1 treatment like IRAK. Therefore, we searched for molecules that can substitute for IRAK in an IL-1 response.

## SUMMARY OF THE INVENTION

The invention provides methods and compositions relating to isolated IRAK3 polypeptides and related polynucleotides having IRAK3-specific structure and activity. The subject IRAK3 polypeptides and polynucleotides can regulate cellular responsiveness to cytokine activation and hence provide important regulators of cell function. The polypeptides may be produced recombinantly from transformed host cells from the subject IRAK3 polypeptide encoding nucleic acids or purified from mammalian cells. The invention provides isolated IRAK3 hybridization probes and primers capable of specifically hybridizing with the disclosed IRAK3 gene, IRAK3-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis (e.g. genetic hybridization screens for IRAK3 transcripts), therapy (e.g. IRAK3 kinase inhibitors to inhibit IL-1 induced signal transduction) and in the biopharmaceutical industry (e.g. as immunogens, reagents for isolating other transcriptional regulators, reagents for screening chemical libraries for lead pharmacological agents, etc.).

## DETAILED DESCRIPTION OF THE INVENTION

The nucleotide sequence of a natural human cDNA encoding a human IRAK3 polypeptide is shown as SEQ ID NO:1, and the full conceptual translate is shown as SEQ ID NO:2. To clone this novel IRAK3 cDNA, we used a the cDNA insert from a mouse EST clone (AA840598, Genome Systems) as a hybridization probe to screen a lambda cDNA library constructed with Phytohemagglutinin-L (PHA-L) activated human peripheral blood leukocytes under low stringent conditions. We isolated a 2.2 kb cDNA clone with an open reading frame encoding 617 amino acids, and determined that the methionine at position 22 of the open reading frame is the first amino acid of the full-length IRAK3 protein, which consists of 596 amino acids with a calculated molecular weight of 67675 daltons. We determined that IRAK3 can function as a signaling molecule for either IL-1 or cytokines that signal through IL-1R related receptors, can bind MyD88 in coexpression and in vitro binding assays, and plays a role in inflammatory responses and/or immune regulation and thus provides a drug target for treatment of inflammatory diseases and immune disorders.

The IRAK3 polypeptides of the invention include incomplete translates of SEQ ID NO:1 which translates and fragments of SEQ ID NO:2 have human IRAK3-specific

amino acid sequence, binding specificity or function. Preferred translates/deletion mutants comprise at least 10, preferably at least 15, more preferably at least 25, more preferably at least 35, most preferably at least 50 consecutive residues of SEQ ID NO:2, preferably of at least one of SEQ ID NO:2, residues 1-99, residues 100-199, residues 200-299, residues 300-399, residues 400-499 and residues 500-596. The subject domains provide IRAK3 domain specific activity or function, such as IRAK3-specific kinase or kinase inhibitory activity, IRAK-3 specific MyD88-binding or binding inhibitory activity, IRAK3 specific antibody binding or binding inhibitory activity.

IRAK3-specific activity or function may be determined by convenient *in vitro*, cell-based, or *in vivo* assays: e.g. *in vitro* binding assays, cell culture assays, in animals (e.g. gene therapy, transgenics, etc.), etc. Binding assays encompass any assay where the molecular interaction of an IRAK3 polypeptide with a binding target is evaluated. The binding target may be a natural intracellular binding target such as an IRAK3 substrate, a IRAK3 regulating protein or other regulator that directly modulates IRAK3 activity or its localization such as MyD88; or non-natural binding target such a specific immune protein such as an antibody, or an IRAK3 specific agent such as those identified in screening assays such as described below. IRAK3-binding specificity may assayed by kinase activity or binding equilibrium constants (usually at least about $10^7 M^{-1}$, preferably at least about $10^8 M^{-1}$, more preferably at least about $10^9 M^{-1}$), by the ability of the subject polypeptide to function as negative mutants in IRAK3-expressing cells, to elicit IRAK3 specific antibody in a heterologous host (e.g a rodent or rabbit), etc. In any event, the IRAK3 binding specificity of the subject IRAK3 polypeptides distinguishes any discernable translation product of EST clone AA840598.

In a particular embodiment, the subject domains provide IRAK3-specific antigens and/or immunogens, especially when coupled to carrier proteins. For example, peptides corresponding to IRAK3-specific domains are covalently coupled to keyhole limpet antigen (KLH) and the conjugate is emulsified in Freunds complete adjuvant. Laboratory rabbits are immunized according to conventional protocol and bled. The presence of IRAK3-specific antibodies is assayed by solid phase immunosorbant assays using immobilized IRAK3 polypeptides of SEQ ID NO:2, see, e.g. Table 2.

Table 2. Immunogenic IRAK3 polypeptides eliciting IRAK3-specific rabbit polyclonal antibody: IRAK3 polypeptide-KLH conjugates immunized per protocol described above.

| IRAK3 Polypeptide Sequence | Immunogenicity |
|---|---|
| SEQ ID NO:2, residues 1-10 | +++ |
| SEQ ID NO:2, residues 12-21 | +++ |
| SEQ ID NO:2, residues 25-37 | +++ |
| SEQ ID NO:2, residues 42-59 | +++ |
| SEQ ID NO:2, residues 62-71 | +++ |
| SEQ ID NO:2, residues 72-85 | +++ |
| SEQ ID NO:2, residues 88-89 | +++ |
| SEQ ID NO:2, residues 105-112 | +++ |
| SEQ ID NO:2, residues 116-122 | +++ |
| SEQ ID NO:2, residues 120-128 | +++ |
| SEQ ID NO:2, residues 175-182 | +++ |
| SEQ ID NO:2, residues 180-195 | +++ |
| SEQ ID NO:2, residues 201-208 | +++ |
| SEQ ID NO:2, residues 213-222 | +++ |
| SEQ ID NO:2, residues 222-230 | +++ |
| SEQ ID NO:2, residues 228-237 | +++ |
| SEQ ID NO:2, residues 230-338 | +++ |
| SEQ ID NO:2, residues 237-245 | +++ |
| SEQ ID NO:2, residues 440-450 | +++ |
| SEQ ID NO:2, residues 442-451 | +++ |
| SEQ ID NO:2, residues 445-452 | +++ |
| SEQ ID NO:2, residues 447-454 | +++ |
| SEQ ID NO:2, residues 449-456 | +++ |
| SEQ ID NO:2, residues 450-457 | +++ |
| SEQ ID NO:2, residues 471-480 | +++ |
| SEQ ID NO:2, residues 495-502 | +++ |
| SEQ ID NO:2, residues 501-510 | +++ |
| SEQ ID NO:2, residues 525-432 | +++ |
| SEQ ID NO:2, residues 527-540 | +++ |

| | |
|---|---|
| SEQ ID NO:2, residues 549-556 | +++ |
| SEQ ID NO:2, residues 563-575 | +++ |
| SEQ ID NO:2, residues 577-590 | +++ |
| SEQ ID NO:2, residues 589-596 | +++ |

The claimed IRAK3 polypeptides are isolated or pure: an "isolated" polypeptide is unaccompanied by at least some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, and more preferably at least about 5% by weight of the total polypeptide in a given sample and a pure polypeptide constitutes at least about 90%, and preferably at least about 99% by weight of the total polypeptide in a given sample. The IRAK3 polypeptides and polypeptide domains may be synthesized, produced by recombinant technology, or purified from mammalian, preferably human cells. A wide variety of molecular and biochemical methods are available for biochemical synthesis, molecular expression and purification of the subject compositions, see e.g. Molecular Cloning, A Laboratory Manual (Sambrook, *et al.* Cold Spring Harbor Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, *et al.*, Greene Publ. Assoc., Wiley-Interscience, NY) or that are otherwise known in the art.

The invention provides binding agents specific to the claimed IRAK3 polypeptides, including substrates, agonists, antagonists, natural intracellular binding targets, etc., methods of identifying and making such agents, and their use in diagnosis, therapy and pharmaceutical development. For example, specific binding agents are useful in a variety of diagnostic and therapeutic applications, especially where disease or disease prognosis is associated with improper utilization of a pathway involving the subject proteins, e.g. NF-κB activation. Novel IRAK3-specific binding agents include IRAK3-specific receptors, such as somatically recombined polypeptide receptors like specific antibodies or T-cell antigen receptors (see, e.g Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory) and other natural intracellular binding agents identified with assays such as one-, two- and three-hybrid screens, non-natural intracellular binding agents identified in screens of chemical libraries such as described below, etc. Agents of particular interest modulate IRAK3 function, e.g. IRAK3-dependent transcriptional activation, esp. dominant negative deletion mutants, etc. Accordingly, the invention also provides methods for modulating signal transduction involving IL-1 receptor activation in a cell comprising the step of modulating IRAK3

kinase activity, e.g. by contacting the cell with a dominant negative IRAK3 deletion mutant, or IRAK3 polynucleotide (below).

The amino acid sequences of the disclosed IRAK3 polypeptides are used to back-translate IRAK3 polypeptide-encoding nucleic acids optimized for selected expression systems (Holler et al. (1993) Gene 136, 323-328; Martin et al. (1995) Gene 154, 150-166) or used to generate degenerate oligonucleotide primers and probes for use in the isolation of natural IRAK3-encoding nucleic acid sequences ("GCG" software, Genetics Computer Group, Inc, Madison WI). IRAK3-encoding nucleic acids used in IRAK3-expression vectors and incorporated into recombinant host cells, e.g. for expression and screening, transgenic animals, e.g. for functional studies such as the efficacy of candidate drugs for disease associated with IRAK3-modulated cell function, etc.

The invention also provides nucleic acid hybridization probes and replication / amplification primers having a IRAK3 cDNA specific sequence comprising SEQ ID NO:1 or fragments thereof, and sufficient to effect specific hybridization thereto (i.e. specifically hybridize with SEQ ID NO:1, particularly in the presence of EST clone AA840598. Such primers or probes are at least 12, preferably at least 24, more preferably at least 36 and most preferably at least 96 bases in length. Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5 x SSPE (0.18 M NaCl, 0.01 M $NaPO_4$, pH7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE; preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE buffer at 42°C. IRAK3 nucleic acids can also be distinguished using alignment algorithms, such as BLASTX (Altschul *et al.* (1990) Basic Local Alignment Search Tool, J Mol Biol 215, 403-410).

Table 3. Exemplary IRAK3 nucleic acids which hybridize with a strand of SEQ ID NO:1 under Conditions I and/or II.

| IRAK3 Nucleic Acids | Hybridization |
|---|---|
| SEQ ID NO:1, nucleotides 1-36 | + |
| SEQ ID NO:1, nucleotides 68-98 | + |
| SEQ ID NO:1, nucleotides 95-130 | + |

| | |
|---|---|
| SEQ ID NO:1, nucleotides 175-220 | + |
| SEQ ID NO:1, nucleotides 261-299 | + |
| SEQ ID NO:1, nucleotides 274-310 | + |
| SEQ ID NO:1, nucleotides 331-369 | + |
| SEQ ID NO:1, nucleotides 530-570 | + |
| SEQ ID NO:1, nucleotides 584-616 | + |
| SEQ ID NO:1, nucleotides 661-708 | + |
| SEQ ID NO:1, nucleotides 689-725 | + |
| SEQ ID NO:1, nucleotides 822-856 | + |
| SEQ ID NO:1, nucleotides 989-1012 | + |
| SEQ ID NO:1, nucleotides 1128-1165 | + |
| SEQ ID NO:1, nucleotides 1238-1258 | + |
| SEQ ID NO:1, nucleotides 1348-1372 | + |
| SEQ ID NO:1, nucleotides 1465-1499 | + |
| SEQ ID NO:1, nucleotides 1562-1593 | + |
| SEQ ID NO:1, nucleotides 1657-1684 | + |
| SEQ ID NO:1, nucleotides 1705-1739 | + |
| SEQ ID NO:1, nucleotides 1822-1861 | + |
| SEQ ID NO:1, nucleotides 1917-1944 | + |
| SEQ ID NO:1, nucleotides 2035-2069 | + |
| SEQ ID NO:1, nucleotides 2161-2183 | + |
| SEQ ID NO:1, nucleotides 2250-2288 | + |

The subject nucleic acids are of synthetic/non-natural sequences and/or are isolated, i.e. unaccompanied by at least some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, preferably at least about 5% by weight of total nucleic acid present in a given fraction, and usually recombinant, meaning they comprise a non-natural sequence or a natural sequence joined to nucleotide(s) other than that which it is joined to on a natural chromosome. Recombinant nucleic acids comprising the nucleotide sequence of SEQ ID NO:1, or fragments thereof, contain such sequence or fragment at a terminus, immediately flanked by (i.e. contiguous with) a sequence other than that which it is joined to on a natural chromosome, or flanked by a native flanking region fewer than 10 kb, preferably fewer than 2 kb, which is at a

terminus or is immediately flanked by a sequence other than that which it is joined to on a natural chromosome. While the nucleic acids are usually RNA or DNA, it is often advantageous to use nucleic acids comprising other bases or nucleotide analogs to provide modified stability, etc.

The subject nucleic acids find a wide variety of applications including use as translatable transcripts, hybridization probes, PCR primers, diagnostic nucleic acids, etc.; use in detecting the presence of IRAK3 genes and gene transcripts and in detecting or amplifying nucleic acids encoding additional IRAK3 homologs and structural analogs. In diagnosis, IRAK3 hybridization probes find use in identifying wild-type and mutant IRAK3 alleles in clinical and laboratory samples. Mutant alleles are used to generate allele-specific oligonucleotide (ASO) probes for high-throughput clinical diagnoses. In therapy, therapeutic IRAK3 nucleic acids are used to modulate cellular expression or intracellular concentration or availability of active IRAK3.

The invention provides efficient methods of identifying agents, compounds or lead compounds for agents active at the level of a IRAK3 modulatable cellular function. Generally, these screening methods involve assaying for compounds which modulate IRAK3 interaction with a natural IRAK3 binding target such as MyD88. A wide variety of assays for binding agents are provided including labeled *in vitro* protein-protein binding assays, immunoassays, cell based assays, etc. The methods are amenable to automated, cost-effective high throughput screening of chemical libraries for lead compounds. Identified reagents find use in the pharmaceutical industries for animal and human trials; for example, the reagents may be derivatized and rescreened in *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development.

*In vitro* binding assays employ a mixture of components including an IRAK3 polypeptide, which may be part of a fusion product with another peptide or polypeptide, e.g. a tag for detection or anchoring, etc. The assay mixtures comprise a natural intracellular IRAK3 binding target. In a particular embodiment, the binding target is an MyD88-derived binding peptide or an IRAK3-derived substrate of IRAK3 kinase activity. While native full-length binding targets may be used, it is frequently preferred to use portions (e.g. peptides) thereof so long as the portion provides binding affinity and avidity to the subject IRAK3 polypeptide conveniently measurable in the assay. The assay mixture also comprises a candidate pharmacological agent. Candidate agents encompass

numerous chemical classes, though typically they are organic compounds; preferably small organic compounds and are obtained from a wide variety of sources including libraries of synthetic or natural compounds. A variety of other reagents may also be included in the mixture. These include reagents like ATP or ATP analogs (for kinase assays), salts, buffers, neutral proteins, e.g. albumin, detergents, protease inhibitors, nuclease inhibitors, antimicrobial agents, etc. may be used.

The resultant mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the IRAK3 polypeptide specifically binds the cellular binding target, portion or analog with a reference binding affinity. The mixture components can be added in any order that provides for the requisite bindings and incubations may be performed at any temperature which facilitates optimal binding. Incubation periods are likewise selected for optimal binding but also minimized to facilitate rapid, high-throughput screening.

After incubation, the agent-biased binding between the IRAK3 polypeptide and one or more binding targets is detected by any convenient way. For IRAK3 kinase assays, 'binding' is generally detected by a change in the phosphorylation of an IRAK3 substrate. In this embodiment, kinase activity may quantified by the transfer to the substrate of a labeled phosphate, where the label may provide for direct detection as radioactivity, luminescence, optical or electron density, etc. or indirect detection such as an epitope tag, etc. A variety of methods may be used to detect the label depending on the nature of the label and other assay components, e.g. through optical or electron density, radiative emissions, nonradiative energy transfers, etc. or indirectly detected with antibody conjugates, etc.

A difference in the binding affinity of the IRAK3 polypeptide to the target in the absence of the agent as compared with the binding affinity in the presence of the agent indicates that the agent modulates the binding of the IRAK3 polypeptide to the IRAK3 binding target. Analogously, in the cell-based assay also described below, a difference in IRAK3-dependent transcriptional activation in the presence and absence of an agent indicates the agent modulates IRAK3 function. A difference, as used herein, is statistically significant and preferably represents at least a 50%, more preferably at least a 90% difference.

The following experimental section and examples are offered by way of

illustration and not by way of limitation.

## EXAMPLES

1. Protocol for at IRAK3 autophosphorylation assay.

A. Reagents:

- Neutralite Avidin: 20 µg/ml in PBS.
- kinase: $10^{-8}$ - $10^{-5}$ M IRAK3 kinase domain at 20 µg/ml in PBS.
- substrate: $10^{-7}$ - $10^{-4}$ M biotinylated IRAK3 substrate at 40 µg/ml in PBS.
- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hour at room temperature.
- Assay Buffer: 100 mM KCl, 10 mM $MgCl_2$, 1 mM $MnCl_2$, 20 mM HEPES pH 7.4, 0.25 mM EDTA, 1% glycerol, 0.5% NP-40, 50 mM BME, 1 mg/ml BSA, cocktail of protease inhibitors.
- [ $^{32}$P]γ-ATP 10x stock: 2 x $10^{-5}$ M cold ATP with 100 µCi [ $^{32}$P]γ-ATP. Place in the 4°C microfridge during screening.
- Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2mM $NaVo_3$ (Sigma # S-6508) in 10 ml of PBS.

B. Preparation of assay plates:

- Coat with 120 µl of stock N Avidin per well overnight at 4°C.
- Wash 2 times with 200 µl PBS.
- Block with 150 µl of blocking buffer.
- Wash 2 times with 200 µl PBS.

C. Assay:

- Add 40 µl assay buffer/well.
- Add 40 µl biotinylated substrate (2-200 pmoles/40 ul in assay buffer)
- Add 40 µl kinase (0.1-10 pmoles/40 ul in assay buffer)
- Add 10 µl compound or extract.
- Add 10 µl [$^{32}$P]γ-ATP 10x stock.
- Shake at 25°C for 15 minutes.
- Incubate additional 45 minutes at 25°C.
- Stop the reaction by washing 4 times with 200 µl PBS.

- Add 150 µl scintillation cocktail.

- Count in Topcount.

D. Controls for all assays (located on each plate):

a. Non-specific binding

b. cold ATP at 80% inhibition.

2. Protocol for high throughput IRAK3-MyD88 binding assay.

A. Reagents:

- Neutralite Avidin: 20 µg/ml in PBS.

- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hour at room temperature.

- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.6, 1 mM $MgCl_2$, 1% glycerol, 0.5% NP-40, 50 mM β-mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors.

- $^{33}$P IRAK3 polypeptide 10x stock: $10^{-8}$ - $10^{-6}$ M "cold" IRAK3 supplemented with 200,000-250,000 cpm of labeled IRAK3 (Beckman counter). Place in the 4°C microfridge during screening.

- Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2mM $NaVO_3$ (Sigma # S-6508) in 10 ml of PBS.

-TRAF2: $10^{-7}$ - $10^{-5}$ M biotinylated MyD88 in PBS.

B. Preparation of assay plates:

- Coat with 120 µl of stock N-Avidin per well overnight at 4°C.

- Wash 2 times with 200 µl PBS.

- Block with 150 µl of blocking buffer.

- Wash 2 times with 200 µl PBS.

C. Assay:

- Add 40 µl assay buffer/well.

- Add 10 µl compound or extract.

- Add 10 µl $^{33}$P-IRAK3 (20-25,000 cpm/0.1-10 pmoles/well = $10^{-9}$ - $10^{-7}$ M final conc).

- Shake at 25°C for 15 minutes.

- Incubate additional 45 minutes at 25°C.

- Add 40 μM biotinylated MyD88 (0.1-10 pmoles/40 ul in assay buffer)
- Incubate 1 hour at room temperature.
- Stop the reaction by washing 4 times with 200 μM PBS.
- Add 150 μM scintillation cocktail.
- Count in Topcount.

D. Controls for all assays (located on each plate):

a. Non-specific binding

b. Soluble (non-biotinylated MyD88) at 80% inhibition.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated polypeptide comprising at least 25 consecutive amino acid residues of the amino acid sequence as set forth as SEQ ID NO:2.

2. An isolated polypeptide comprising at least 50 consecutive amino acid residues of the amino acid sequence as set forth as SEQ ID NO:2.

3. An isolated polypeptide according to claim 1, wherein said polypeptide has an activity selected from at least one of: a MyD88-binding or binding inhibitory activity, and an IRAK3-specific antigenicity..

4. An isolated polynucleotide comprising at least 24 consecutive nucleotides of SEQ ID NO:1.

5. The polynucleotide of claim 4, comprising at least 36 consecutive nucleotides of SEQ ID NO:1.

6. The polynucleotide of claim 4, comprising at least 48 consecutive nucleotides of SEQ ID NO:1.

7. A recombinant or isolated polynucleotide encoding a polypeptide according to claim 1.

8. A cell comprising a polynucleotide according to claim 4.

9. A method of making an isolated polypeptide comprising SEQ ID NO:2, or at least a 25 residue fragment thereof, said method comprising steps: introducing a polynucleotide according to claim 4 into a host cell or cellular extract, incubating said host cell or extract under conditions whereby said nucleic acid is expressed as a transcript and said transcript is expressed as a translation product comprising said polypeptide, and isolating said translation product.

10. A method of screening for an agent which modulates the interaction of a IRAK3 polypeptide to a binding target, said method comprising the steps of:

incubating a mixture comprising:

an isolated polypeptide according to claim 1,

a binding target of said polypeptide, and

a candidate agent;

under conditions whereby, but for the presence of said agent, said polypeptide specifically binds said binding target at a reference affinity;

detecting the binding affinity of said polypeptide to said binding target to determine an agent-biased affinity,

wherein a difference between the agent-biased affinity and the reference affinity indicates that said agent modulates the binding of said polypeptide to said binding target.

11. The method of claim 10, wherein said binding target is a natural intracellular substrate and said reference and agent-biased binding affinity is detected as phosphorylation of said substrate.

12. The method of claim 10, wherein said binding target is an MyD88 derived peptide.

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Ser Glu Lys Ser Tyr Gln Glu Gly Gly Phe Pro Asn Ile Leu Phe Lys
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gaa aca gcc aat gtc acc gtg gat aat gtt ctt att cct gaa cat aat   492
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Val Ile Cys Gly Ser Ile Ser Ser Ala Asn Ile Leu Leu Asp Asp Gln
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Tyr Lys Lys Glu
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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/18800

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 194, 7.1, 7.2, 252.3, 320.1; 536/23.5; 530/350, 351; 514/2; 424/85.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Sequence search on MSPAR

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | Database Geneseq35 on Maspar, Novartis AG, (*Great Britain) No. W47023, DE VRIES et al. 'Arabidopsis thaliana SERK protein' 20 November 1997, see sequence alignment. | 1-12 |
| A | Database Geneseq35 on Maspar, TULARIK INC. (United States) No. W14306, CAO et al. 'Interleukin-1 Receptor- associated protein kinase' 09 January 1997, see sequence alignment. | 1-12 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "B" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>20 DECEMBER 1999 | Date of mailing of the international search report<br>12 JAN 2000 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>GARNETTE D. DRAPER<br>Telephone No. (703) 308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/18800

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | Database Geneseq35 on Maspar, Sumitomo Chem. Co. LTD. (Japan), No. W00628, 'Protein kinase #1 containing sequence from Solanaceae solanum' 23 April, 1996, see sequence alignment. | 1-12 |
| A | Database Geneseq35 Maspar (United States) No. A49332, MARTIN et al. 'Map-based cloning of a protein kinase gene conferring disease resistance in tomato' 1993, see sequence alignment. | 1-12 |
| A | Database Geneseq35 on Maspar, (United States), No. S38326, MORAN et al. 'Molecular cloning of two novel protein kinase genes from Arabidopsis thaliana' 13 January 1995, see sequence alignment. | 1-12 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/18800

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7): C12N 15/00, 15/54, 15/01, 15/63; G01N 33/50, 33/53; C07K 1/00, 14/52, 14/705; A61K 38/16, 38/46

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/69.1, 194, 7.1, 7.2, 252.3, 320.1; 536/23.5; 530/350, 351; 514/2; 424/85.1